

Article

Identification of *Escherichia* spp. strains in street-vended beverages and associated preparation surfaces using 16S rRNA analysis

Chong, S.Y., Rao, P.V. and Soon, Jan Mei

Available at <http://clock.uclan.ac.uk/19905/>

Chong, S.Y., Rao, P.V. and Soon, Jan Mei ORCID: 0000-0003-0488-1434 (2017) Identification of Escherichia spp. strains in street-vended beverages and associated preparation surfaces using 16S rRNA analysis. International Food Research Journal, 24 (4). pp. 1811-1818. ISSN 1985-4668

It is advisable to refer to the publisher's version if you intend to cite from the work.

For more information about UCLan's research in this area go to <http://www.uclan.ac.uk/researchgroups/> and search for <name of research Group>.

For information about Research generally at UCLan please go to <http://www.uclan.ac.uk/research/>

All outputs in CLoK are protected by Intellectual Property Rights law, including Copyright law. Copyright, IPR and Moral Rights for the works on this site are retained by the individual authors and/or other copyright owners. Terms and conditions for use of this material are defined in the [policies](#) page.

**Identification of *Escherichia* spp. strains in street-vended beverages
and associated preparation surfaces using 16S rRNA analysis**

¹Chong, S. Y., ¹Rao, P. V., ^{2*}Soon, J. M.

¹Faculty of Agro-Based Industry, Universiti Malaysia Kelantan Jeli Campus,
17600 Jeli, Kelantan, Malaysia

²International Institute of Nutritional Sciences and Applied Food Safety
Studies, School of Sport and Wellbeing, University of Central Lancashire,
Preston, PR1 2HE UK

*corresponding author: jmsoon@uclan.ac.uk; Tel: (+44)1772 89 4567

13 **Abstract**

14 Street-vended beverages are commonly prepared by mechanical extraction
15 of the pulp, usually unpasteurised and requires multiple handling steps.
16 Foodborne pathogens transmitted via skin of street vendors or via faecal-oral
17 route may contaminate the preparation surfaces and beverages. The aim of
18 this study is to identify *Escherichia* spp. strains of street-vended beverages
19 and their associated preparation surfaces using 16s rRNA analysis. The
20 hygienic practice of vendors was represented by *Staphylococcus* spp.
21 analysis and *Staphylococcus aureus* is not detected in beverages and
22 associated preparation surfaces. A total of 80 samples (18 beverages, 15
23 swab samples and 47 direct film samples) were collected followed by
24 enumeration of microbial load. Polymerase Chain Reaction (PCR)
25 amplification and 16S ribosomal ribonucleic acid (rRNA) sequencing were
26 carried out. Results of 16S rRNA sequence analysis indicated that three
27 gram-negative isolates were identified as *Escherichia coli* RM9387
28 (Accession no. CP009104.1), *Escherichia coli* c164 (Accession no.
29 JQ781646.1) and *Escherichia fergusonii* E10 (Accession no. KJ626264.1)
30 with similarity value of 99% respectively.

31

32 **Keywords:** coconut water, *Escherichia fergusonii*, hygiene, *Staphylococcus*
33 *aureus*, sugarcane juice

34

35 **Introduction**

36 In developing countries, beverages sold by street-vendors are widely
37 consumed by the society (Al Mamun *et al.*, 2013). In urban as well as rural
38 area, street-vended consumables are becoming popular as they are
39 inexpensive, convenient and attractive (World Health Organisation, 1996).
40 Besides, such beverages are readily available and affordable to the
41 population (Mahale *et al.*, 2008). The World Health Organization (WHO)
42 defined street-vended foods as “foods and beverages prepared and/or sold
43 by vendors in streets and other public places for immediate consumption or
44 consumption at a later time without further processing or preparation” (WHO,
45 1996). Unpasteurised clouded, untreated juices are widely consumed
46 because of the satisfactory flavour attributes. For instance, coconut water
47 and sugarcane juice can be easily prepared by mechanical extraction of the
48 pulp and liquid of mature fruits (Nonga *et al.*, 2014).

49 In Malaysia, the preponderance of foodborne disease as an important
50 public health problem is alarmingly high as it causes significant impact on
51 economic and trade of the country (Sharifa *et al.*, 2013). Based on the current
52 society, there are a few phenomenon that are detected and encountered.
53 The consumers emphasize on sensory taste instead of the hygienic level of
54 beverages prepared by vendors. Furthermore, the contamination of
55 beverages and unhygienic preparation surfaces that leads to foodborne
56 outbreaks and will harm the safety of consumers. Sources of contamination
57 may vary (Tambekar *et al.*, 2009) and pathogenic organisms are able to enter
58 and contaminate foods and beverages through damaged or injured plant and
59 fruit surfaces that occur during growing, harvesting as well as improper

60 washing during preparation. Such beverages are identified as potential
61 sources of bacterial pathogens notably coliform, *Escherichia coli*, and
62 *Staphylococcus aureus* (Mukhopadhyay *et al.*, 2011). Tan *et al.* (2013)
63 reported that in Malaysia, transmission of bacteria such as *S. aureus* into
64 food is mainly due to the unhygienic practice of food handlers.

65 Various types of pathogenic microbes in water source including bacteria,
66 viruses, and protozoa have been linked to human disease caused by the
67 usage of contaminated food and water (Kinge *et al.*, 2008). These water-
68 related diseases include diarrhoea, typhoid, hepatitis, cholera, and dysentery
69 (Kinge *et al.*, 2008). In developing countries, limited access to safe water and
70 substandard sanitation continues to jeopardise human health. According to
71 WHO (2003), since then, pathogenic bacteria have been identified as the
72 utmost etiological agent in the vast majority of the waterborne outburst
73 reported worldwide. Hence a study of the street-vended beverages was
74 undertaken to assess the safety for human consumption and as possible
75 sources of bacterial pathogens.

76

77 **Materials and Methods**

78

79 *Sampling*

80 Fresh, unpasteurised sugarcane juice (n=9) and coconut water samples
81 (n=9) were collected from street vendors in Jeli District, Malaysia. All samples
82 were stored below 4°C and transported to the laboratory for microbiological
83 analyses. The pH values of all sampled beverages were measured.

84

85 *Swabbing of preparation surfaces*

86 Samples were collected from vendors' left and right palm (72 cm² on
87 each palm) using sterile 3M swabs pre-moistened with 0.1% sterile peptone
88 water (Oxoid, England). The surface points (upper pressing unit, lower
89 pressing unit) (100 cm²) of sugarcane extractor, coconut opener (54 cm²)
90 and apron (100 cm²) were swabbed. Swabbing was performed horizontally
91 and vertically (Harrigan, 1998). In addition, Sanita-kun (JNC Corporation,
92 Japan) direct stamping was also carried out on all preparation surfaces. Then,
93 the swabs and Sanita-kun film samples were stored and transported to
94 laboratory under cold-storage conditions (Tan *et al.*, 2014).

95

96 *Bacteriological Examination*

97 Total Plate Counts (TPCs) were determined using plate count agar
98 (Oxoid, England) pour plate method and incubated at 37°C for 24 hours.
99 Visible colonies were counted using Bacterial Colony Counter (Funke Gerber,
100 Germany) (Harrigan, 1998). The existence of coliform was determined using
101 multiple-tube fermentation technique and the three-tube most-probable-
102 number (MPN) method. Appropriate dilutions of samples were inoculated into
103 triplicates of Lauryl Sulfate Tryptose broth (Merck, Germany) (Bagci and
104 Temiz, 2011). Then, one loopful of culture from presumptive positive Lauryl
105 Sulfate Tryptose tubes were confirmed by sub-culturing into Brilliant Green
106 Bile broth (R and MJL Chemicals, U.K.) and further incubated at 37°C for 24
107 hours (Bagci and Temiz, 2011).

108

109 *Determination of Escherichia coli*

110 One loopful of positive Lauryl Sulfate Tryptose broth were transferred
111 into EC broth (Oxoid, England) and incubated. Presumptive positive tubes
112 were further isolated on Eosin Methylene Blue agar (Merck, Germany)
113 (Mallmann and Darby, 1941) and subjected to IMViC test. For indole
114 production, 0.3 ml of Kovác's reagent (Merck, Germany) were added into
115 incubated tryptone broth (Merck, Germany) containing presumptive *E. coli*
116 colony (Tan *et al.*, 2014). Indole positive colony from nutrient agar was
117 inoculated on Simmons Citrate agar (Oxoid, England) slant for citrate test.

118

119 *Determination of Staphylococcus aureus*

120 For each dilution to be plated, 1 ml of inoculum was aseptically
121 distributed equitably to 3 plates of Baird-Parker agar (Merck, Germany) with
122 Egg Yolk Tellurite Emulsion (Oxoid, England).
123 Presumptive *Staphylococcus aureus* colonies from Baird-Parker agar were
124 transferred into 0.2 ml Brain Heart Infusion broth (Merck, Germany) and
125 emulsified thoroughly for coagulase test. Then, 0.3 ml of rabbit coagulase
126 plasma with EDTA (Remel, United Kingdom) were added to Brain Heart
127 Infusion culture and examined periodically for clot formation (Harrigan, 1998;
128 Kateete *et al.*, 2010).

129

130 *DNA extraction and agarose gel electrophoresis*

131 Presumptive positive *E. coli* samples were preserved using glycerol
132 stock. Bacteria cells were then inoculated into 5 ml Luria Bertani broth (Merck,
133 Germany) and incubated overnight until the optical density (OD) value of
134 culture falls within the range of 0.8-1.0 at 600 nm (Kinge *et al.*, 2008; Magray
135 *et al.*, 2011). Genomic DNA were then extracted (Fattahi *et al.*, 2013) using
136 G-spin Total Genomic DNA Kit (Intron, Korea). Horizontal agarose 1% w/v
137 slab gel was prepared using agarose (Vivantis, USA), 1X Tris-Acetate-EDTA
138 (40 mM tris-acetate; 20 mM glacial acetic acid and 1.0 mM EDTA, pH 8.0)
139 and 10 µl of 1 mg/ml ethidium bromide (Kinge *et al.*, 2008). DNA ladder of 1
140 kb and 100 bp and each DNA template with loading dye (Vivantis, USA) were
141 loaded into the well prior to electrophoresis. The agarose gel was
142 electrophoresed for 45 minutes at 80 V and EtBr-stained agarose gel was
143 visualised under UV transilluminator (Kazemi and Hajizadeh, 2012).

144

145 *Polymerase Chain Reaction (PCR)*

146 Extracted DNA from isolated bacteria were then used for PCR and 16S
147 rRNA analysis using 16S rRNA universal primers (Forward primer 5'-
148 AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-
149 CGGCTACCTTGTACTT-3') (Magray *et al.*, 2011). PCR reaction was
150 carried out in 50 µl PCR reaction mixture (2.5 U Taq DNA polymerase, 10 p
151 moles each of the forward and reverse primers, 1X Taq buffer, 0.2 mM dNTP
152 mix, 1.5 mM MgCl₂, DNA template and sterile distilled water) (1st Base,
153 Singapore). PCR amplification was performed in a Thermal Cycler
154 (Eppendorf AG 22331 Hamburg, Germany) under conditions such as heat

155 denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at
156 94°C for 1 minute; annealing at 59°C for 1 minute and extension at 72°C for
157 1 minute. This was followed by a final extension step at 72°C for 7 minutes
158 and 4°C hold (Kinge *et al.*, 2008; Pathak *et al.*, 2013). Also, the purity of DNA
159 were determined using Nanodrop Spectrophotometer (DeNovix, United
160 State) at $A_{260/280}$ and $A_{260/230}$ (Pathak *et al.*, 2013). Vivantis Gel DNA
161 Purification Kit was used to perform gel extraction purification.

162

163 *16S rRNA Sequencing*

164 Purified PCR products were subjected for 16S rRNA fragment
165 sequencing. The relative molecular sizes of the PCR products were
166 compared against the sequences from the Gen Bank database, National
167 Centre for Biotechnology Information (NCBI) using Basic Local Alignment
168 Search Tool (BLAST) analysis program (Fattahi *et al.*, 2013).

169

170 **Results and Discussion**

171

172 The microbiological standard of beverages and preparation surfaces
173 concerned were compared against Ministry of Health (MOH) Malaysia
174 drinking water quality as well as World Health Organisation (WHO), U.S.
175 Environmental Protection Agency (EPA) and Food and Drug Administration
176 (FDA) drinking water regulations. Prior to any microbial test, the pH of
177 sugarcane juice were taken as it may contribute to microbial growth. The pH
178 of sugarcane juice were recorded from stall A (pH 4.38), stall B (pH 5.35) and

179 stall C (pH 4.67). These values are in the range of Oliveira *et al.* (2006) and
180 Mahale *et al.* (2008) studies (pH 4.89). Although low pH value may have
181 inhibitory effects to microbial contamination (Nonga *et al.*, 2014) and the
182 survival of pathogenic bacteria in beverages, large groups of bacteria are
183 able to grow at pH greater than 4 (Oliveira *et al.*, 2006).

184 Based on the distribution of bacteria from total plate counts of sugarcane
185 juice, stall B showed the highest bacteria load at 2.63 ± 0.01 log CFU/ml
186 ($P < 0.05$) compared to Stall A and C's sugarcane juice (Table 1). The
187 detection of coliform contamination in juice samples clearly indicated that it
188 does not fulfil WHO and EPA's standard where no coliform should be
189 detected. All sugarcane juice samples recorded 240 MPN/ml for presumptive
190 *Escherichia coli* counts (ECCs). Finding presumptive pathogenic *E. coli*
191 species in beverages ready for consumption at all three stalls is generally
192 viewed as an indication of faecal contamination (Lambrechts *et al.*, 2014).

193 *Staphylococcus aureus* had been reported in street-vended juice
194 samples (Suneetha *et al.*, 2011). In this study, stall A sugarcane juice
195 reported the highest staphylococci load (2.62 ± 0.02 log CFU/ml) (Table 1). In
196 contrast, staphylococci were not detected in *S. aureus* selective agar in stall
197 C's sugarcane juice sample. This indicated better hygiene management of
198 beverages preparation since human skin and mucous membrane are the
199 natural carrier of *S. aureus* (Lambrechts *et al.*, 2014). Based on the
200 coagulase test, none of the samples from stall A and stall B sugarcane juice
201 showed coagulation. The possible species on Baird-Parker agar but
202 responded as negative-coagulase is *Staphylococcus epidermidis*.

203 For coconut water, Yong *et al.* (2009) claimed that the pH of fresh
204 coconut water for consumption should fall within the range of 4.6–5.6. The
205 present study showed the average pH of coconut water from stalls A, B and
206 C were 4.80, 5.27 and 4.78 respectively which is compatible with the study.
207 Coliform counts in coconut water ranged between 43 MPN/ml to >1000
208 MPN/ml (Table 1) which is also incompatible with WHO and EPA standard
209 as no coliform should be present. The presumptive positive *E. coli* species
210 detected in coconut water was non-pathogenic Enterobacteriaceae bacteria.
211 For identification of *Staphylococcus*, stall A recorded the highest
212 staphylococci bacteria load followed by stall B while stall C's samples did not
213 show characteristic feature of staphylococci colony.

214 Pearson correlation showed that the average TPCs with presumptive
215 *Staphylococcus aureus* counts (SACs) of sugarcane juice and coconut water,
216 from all three selected vendors were highly correlated, $r=0.861$. It delineated
217 that causal relationship exist between TPC and ECC of beverages as well as
218 among TCC and SAC of beverages collected from all three stalls.
219 *Staphylococcus* counts were detected in 60% (9/15) of the associated
220 preparation surfaces and 26.67% (4/15) of *E. coli* counts were detected on
221 the examined preparation surfaces using Sanita-kun direct stamping method.
222 The linear correlation coefficient between the Baird-Parker agar
223 *Staphylococcus aureus* counts (SACs) and Sanita-kun *Staphylococcus*
224 *aureus* counts (SSCs) method was $r=0.956$ which showed high correlation.
225 Morita *et al.* (2003) and Park and Kim (2013) reported that the plate count

226 and Sanita-kun film count correlation coefficient were $r=0.99$ and therefore
227 Sanita-kun is an acceptable alternative to various agar medium.

228 Table 2 showed that there were no significant difference ($P>0.05$) in
229 TPCs between both left and right hands of the street vendors. The TPCs of
230 stall B handler's left and right hands recorded the highest bacteria load at
231 2.96 ± 0.02 and 2.90 ± 0.02 log CFU/ml respectively. Stall B's handler also
232 recorded the highest *Staphylococcus* sp. count for sugarcane juice
233 (2.63 ± 0.01 log CFU/ml) and coconut water (2.56 ± 0.01 log CFU/ml). The
234 TPCs on both hands were compatible with Christison *et al.* (2008) who stated
235 that the TPCs of handlers' hands were comparably higher than beverages.
236 Likewise, similar trends were observed for stall A and stall C vendor's hands.
237 It can be deduced that cross-contamination occurs where partial microbial
238 load was transmitted from the preparation surface during handling process
239 (Mahale *et al.*, 2008) and from handler's hands to the sugarcane juice and
240 coconut water (Tan *et al.*, 2014).

241 Among all five types of preparation surfaces examined, sugarcane
242 extractors revealed the highest TPCs (4.13 ± 0.01 and 3.91 ± 0.01 log CFU/ml)
243 for stall B and stall C respectively (Table 2). Gilbert *et al.* (2000) suggested
244 that several factors attributed to the prevalence of coliform on preparation
245 surfaces i.e. the unhygienic practices of vendors, equipment design (e.g. the
246 uneven and ragged surface of the upper and lower pressing unit of
247 sugarcane extractor that build-up food debris and microorganisms) and
248 cleaning and sanitation procedures. Results from the present study showed

249 that sugarcane juice extractor from stall C exhibited positive citrate test with
250 the highest presumptive *E. coli* counts (240 MPN/ml, 2.79 ± 0.01 log CFU/cm²).

251 The microbial status of the coconut opener which is a knife used by
252 street vendors is also shown in Table 2. The total coliform counts and
253 presumptive *Escherichia coli* MPN test results were negative for all coconut
254 opener samples analysed. The microbiological quality of street vendors'
255 aprons with TPCs ranged from < 2.40 to 3.77 ± 0.02 log CFU/ml. There is a
256 vast difference between the total microbial load of stall B vendor's apron
257 (3.77 ± 0.02 log CFU/ml) compared with stall A and stall C (Table 2).

258 This study showed high bacterial load in beverages ranging from
259 2.12 ± 0.01 to 2.63 ± 0.01 log CFU/ml. The TPCs for preparation surfaces
260 fluctuates and covers a wide range, i.e. from an acceptable range of < 2.40
261 to 4.13 ± 0.01 log CFU/ml. This reflects the inadequate sanitary of the
262 sugarcane juice, coconut water and utensils used (Oliveira *et al.* 2006).
263 Results showed that different street vendors will significantly affect ($P < 0.05$)
264 the presumptive *Staphylococcus aureus* load for each preparation surfaces.
265 The staphylococci microbial load for left and right hands, sugarcane extractor
266 and aprons using both BPA and Sanita-kun *S. aureus* direct stamping
267 method were in the order of stall B > stall A > stall C. However, all preparation
268 surfaces were tested as coagulase negative and eliminated the possibility of
269 the presence of pathogenic *Staphylococcus aureus* species.

270 High surrounding temperature of street-vended beverages favours
271 bacteria growth (Nonga *et al.* 2014). Furthermore, heavy vehicular traffic also
272 contributes to high TPCs of street-vended stalls (Mugampoza *et al.*, 2013)

273 and are susceptible to contamination by dust-laden microorganisms. With
274 regards to waste disposal, dumping of wastes on-site with piles of uncovered
275 waste is another unhygienic source that causes further cross-contamination
276 to the consumable goods (Nonga *et al.*, 2014).

277 In some circumstances, both swabbing and dry culture medium methods
278 have pros and cons. The direct stamping method using sheet medium can
279 effectively pick up bacteria present at the surface examined. While for sterile
280 swabs, the collection surface is notably smaller and if excess moisture is
281 applied on the sampling surface, it can cause residual to remain on the tested
282 surface. However, swab samples are best suited for use on irregular surfaces
283 such as sugarcane extractor in this study while flat sheet medium is less
284 preferred. The Sanita-kun *E. coli* and coliform chromogenic medium were
285 metabolised by the growing microorganism. The coloured colonies were
286 easily distinguished between *E. coli* and other coliform colonies because the
287 dry medium was hydrolysed by galactosidase from coliforms and will produce
288 X-Gal visible blue dye upon coliform growth. Meanwhile, salmon-glucuronic
289 acid composed in the medium was hydrolysed by glucuronidase from *E.*
290 *coli* to produce a red-purple dye (Ushiyama and Iwasaki, 2010). The Sanita-
291 kun *Staphylococcus aureus* dry culture medium contains chromogenic agent
292 and a specific fluorescence enzyme substrate (Waku *et al.* 2013). A buffering
293 agent is also present to adjust and maintain the pH at approximately 7.0 to
294 favour the growth of bacteria. As bacteria grow, the blue and black colour
295 colonies will develop. This eases the direct enumeration of *S. aureus*
296 compared with enumeration on Baird-Parker agar where *S. aureus* was

297 distinguished among black colonies by identifying opaque ring clear zones
298 (Waku *et al.*, 2013).

299 DNA extractions were conducted on *E. coli* strains isolated from
300 sugarcane juice of all stalls and sugarcane extractor from stall C as only
301 replicates from these four isolates were identified as pathogenic *E. coli*
302 through IMViC test. Replicates of extracted DNA samples were subjected to
303 gel electrophoresis. All DNA samples migrated from the cathode towards the
304 anode, showing bands of DNA fragments at respective wells while elution
305 without DNA samples was set as the negative control. The rate of travel of
306 DNA towards the positive pole of agarose gel varies inversely with its
307 molecular weight. This is explained by the large size of DNA fragments
308 entangled in the gel matrix and which migrated slowly (Magray *et al.*, 2011).
309 Since the initial DNA extracts often contains RNA and protein, it caused
310 smearing of bands and may interfere with the extracted DNA and need to be
311 purified to retain only targeted DNA fragment. DNA products of all samples
312 were subjected to PCR to multiply the copies of DNA from a single strand of
313 DNA extracted. Through PCR, the targeted DNA was amplified to enable the
314 selection of *E. coli*. Universal primers were used for the amplification of 16S
315 rRNA gene fragment (Magray *et al.*, 2011).

316 The negative control in the above agarose gel for gel electrophoresis of
317 PCR product received the PCR mixture containing no DNA template (Fattahi
318 *et al.*, 2013). High intensity of PCR products in Figure 1 indicates that
319 selective amplification of *E. coli* was successfully attained using universal
320 primers during annealing at 72°C with high concentration of DNA fragments

321 obtained (Sabat *et al.*, 2000). From the gel electrophoresis of PCR products,
322 only PCR products with high intensity and clear band under UV illumination
323 (S3, S4, S6 and S9) from each type of sample were selected for PCR
324 purification.

325 Purification step was performed with the aid of Vivantis Gel DNA
326 Recovery Kit for rapid purification of residual reagents during PCR reaction
327 and elimination of the unspecific DNAs that caused smearing of bands
328 observed in S3, S4, S6 and S9. The clear and single sharp purified bands of
329 1500 bp (Figure 2) represents good quality DNA and these enable the 16S
330 rRNA fragments (Fattahi *et al.*, 2013) of samples from sugarcane juices and
331 sugarcane juice extractor to be subjected to DNA sequencing to identify
332 strains of *Escherichia coli*.

333 The sequencing results were obtained from MyTACG Bioscience
334 Enterprise. All four isolates were aligned using nucleotide BLAST to
335 ascertain the sequence similarity against the NCBI database. Based on the
336 results obtained, the genera with highest occurring frequency are
337 *Escherichia* spp. in all four samples (Table 3).

338 *Escherichia coli* strain RM9387 was previously linked to contaminated
339 milk and is commonly isolated from cattle faeces (Yan *et al.* 2015). *E. coli*
340 RM9387 were also associated with recreational and municipal drinking water
341 outbreaks (Kaper *et al.* 2004). *Escherichia fergusonii*, isolated from stall C's
342 sugarcane juice extractor is the closest related species to *Escherichia coli*
343 and both has been reported as pathogenic species in *Enterobacteriaceae*.
344 *Escherichia fergusonii* and *Escherichia coli* can be isolated from faeces and

345 water samples (Maheux *et al.* 2014). *Escherichia fergusonii* had recently
346 been shown to cause disease in animals and humans (Forgetta *et al.* 2012)
347 as it can influence the intestinal function of both poultry and mammals
348 (Hariharan *et al.* 2007). The presence of *E. coli* on sugarcane plant could be
349 due to contamination from animals' or pests' faeces (Duncan and Colmer
350 1964; Heaton and Jones 2008) instead of cross-contamination from handlers
351 as *E. coli* were not detected on handlers' hands or aprons. Furthermore,
352 when transporting the sugarcane plant, the exposed ends of sugarcane plant
353 can be a favourable environment for the multiplication of *E. coli*.

354

355 **Conclusion**

356

357 *Escherichia coli* and *Staphylococcus* spp. were present in juice samples
358 and some preparation surfaces. The highest total plate count was recorded
359 in both sugarcane juice and coconut water from stall B. The detection of *E.*
360 *coli* strain RM9387 in stalls A and B sugarcane juice, *E. coli* strain c164 in
361 stall C's sugarcane juice sample and *E. fergusonii* strain E10 in stall C
362 sugarcane juice extractor showed that contamination of street-vended
363 beverages or preparation surfaces may present health risk and leads to
364 foodborne illnesses.

365 Street-vended beverages are prepared for immediate consumption and
366 are usually untreated. The results of this study demonstrated that beverages
367 extracted from fresh fruits and associated preparation surfaces are identified
368 as potential sources of bacterial pathogens. The isolated pathogenic *E. coli*

369 strains may present health risk and leads to foodborne illnesses. Accordingly,
370 adoption of strict hygienic measures should be followed during the
371 preparation of street-vended beverages. Regular monitoring of the quality of
372 street-vended beverages is vital to avoid foodborne illnesses. The public
373 need to have greater awareness with respect to food safety while the
374 authority should enforce regulations by making basic Food Handling Course
375 compulsory to food handlers and further training as required by the relevant
376 authority. Associated preparation surfaces such as sugarcane extractor and
377 coconut opener are potential sources of contamination and should be
378 cleaned and sanitised regularly.

379

380 **References**

381

382 Al Mamun, M., Rahman, S. M. M. and Turin, T. C. 2013. Microbiological
383 quality of selected street food items vended by school-based street food
384 vendors in Dhaka, Bangladesh. *International Journal of Food Microbiology*
385 166(3): 413-418.

386

387 Bagci, U. and Temiz, A. 2011. Microbiological quality of fresh-squeezed
388 orange juice and efficacy of fruit surface decontamination methods in
389 microbiological quality. *Journal of Food Protection* 74(8): 1238–1244.

390

391 Christison, C. A., Lindsay, D. and von Holy, A. 2007. Cleaning and handling
392 implements as potential reservoirs for bacterial contamination of some ready-

393 to-eat foods in retail delicatessen environments. Journal of Food Protection
394 70(12): 2878–2883.

395

396 Duncan, C. L. and Colmer, A. R. 1964. Coliforms associated with sugarcane
397 plants and juices. Applied Microbiology 12(2): 173–177.

398

399 Fattahi, F., Mirvaghefi, A., Farahmand, H., Rafiee, G. and Abdollahi, A. 2013.
400 Development of 16S rRNA targeted PCR method for the detection of
401 *Escherichia coli* in rainbow trout (*Oncorhynchus mykiss*). Iranian Journal of
402 Pathology 8(1): 36–44.

403

404 Forgetta, V., Rempel, H., Malouin, F., Vaillancourt, R., Topp, E., Dewar, K.
405 and Diarra, M. S. 2012. Pathogenic and multidrug-resistant *Escherichia*
406 *fergusonii* from broiler chicken. Poultry Science 91(2): 512-525.

407

408 Gilbert, R. J., de Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C. D.,
409 Richards, S., Roberts, D. and Bolton, F. J. 2000. Guidelines for the
410 microbiological quality of some ready-to-eat foods sampled at the point of
411 sale. PHLS Advisory Committee for Food and Dairy Products.
412 Communicable Disease and Public Health / PHLS 3(3): 163–167.

413

414 Hariharan, H., López, A., Conboy, G., Coles, M. and Muirhead, T. 2007.
415 Isolation of *Escherichia fergusonii* from the faeces and internal organs of a
416 goat with diarrhea. Canadian Veterinary Journal 48(6): 630.

417

418 Harrigan, W. F. 1998. Laboratory Methods in Food Microbiology. 3rd ed.
419 California, USA: Academic Press.

420

421 Heaton, J. C. and Jones, K. 2008. Microbial contamination of fruit and
422 vegetables and the behaviour of enteropathogens in the phyllosphere: a
423 review. Journal of Applied Microbiology 104(3): 613–626.

424

425 Kaper, J. B., Nataro, J. P. and Mobley, H. L. 2004. Pathogenic *Escherichia*
426 *coli*. Nature Reviews Microbiology 2(2): 123-140.

427

428 Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S.,
429 Nanteza, A., Joloba, M. L. and Najjuka, F. C. 2010. Identification of
430 *Staphylococcus aureus*: DNase and mannitol salt agar improve the efficiency
431 of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials
432 9: 23. <http://doi.org/10.1186/1476-0711-9-23>

433

434 Kazemi, M. and Hajizadeh, H. S. 2012. An efficient protocol for genomic DNA
435 extraction from *Alcea rosea*. Journal of Agricultural Technology 8(1): 319–
436 326.

437

438 Kinge, C. N. W., Mbewe, M. and Sithebe, N. P. 2008. Detection of bacterial
439 pathogens in river water using multiplex-PCR. In Hernandez-Rodriguez, P.
440 (Ed.). Polymerase Chain Reaction, p. 531-554. Rijeka: InTech.

441

442 Lambrechts, A., Human, I., Doughari, J. and Lues, J. F. R. 2014. Bacterial
443 contamination of the hands of food handlers as indicator of hand washing
444 efficacy in some convenient food industries. Pakistan Journal of Medical
445 Sciences 30(4): 755–758.

446

447 Magray, M. S. U. D., Kumar, A., Rawat, A. K. and Srivastava, S. 2011.
448 Identification of *Escherichia coli* through analysis of 16S rRNA and 16S-23S
449 rRNA internal transcribed spacer region sequences. Bioinformation 6(10):
450 370–371.

451

452 Mahale, D. P., Khade, R. G. and Vaidya, V. K. 2008. Microbiological analysis
453 of street vended fruit juices from Mumbai city , India. Internet Journal of Food
454 Safety 10: 31–34.

455

456 Maheux, A. F., Boudreau, D. K., Bergeron, M. G. and Rodriguez, M. J. 2014.
457 Characterization of *Escherichia fergusonii* and *Escherichia albertii* isolated
458 from water. Journal of Applied Microbiology 117(2): 597-609.

459

460 Mallmann, W. L. and Darby, C. W. 1941. Uses of a lauryl sulfate tryptose
461 broth for the detection of coliform organisms. American Journal of Public
462 Health and the Nation's Health 31(2):127–134.

463

464 Morita, H., Ushiyama, M., Aoyama, S. and Iwasaki, M. 2003. Sensitivity and
 465 specificity of the Sanita-Kun aerobic count: Internal validation and
 466 independent laboratory study. Journal of AOAC International 86(2): 355-366.
 467

468 Mugampoza, D., Byarugaba, G. W. B., Nyonyintono, A., Nakitto, P., Campus,
 469 B. and Kingdom, U. 2013. Occurrence of *Escherichia coli* and *Salmonella*
 470 spp . in street-vended foods and general hygienic and trading practices in
 471 Nakawa Division , Uganda. American Journal of Food and Nutrition 3: 167–
 472 175.
 473

474 Mukhopadhyay, M., Majumdar, M. and Basu, P. 2011. Microbial
 475 contamination of street vended fruit juices in kolkata city. Internet Journal of
 476 Food Safety 13: 1–5.
 477

478 Nonga, H. E., Simforian, E. A. and Ndabikunze, B. K. 2014. Assessment of
 479 physicochemical characteristics and hygienic practices along the value chain
 480 of raw fruit juice vended in Dar es Salaam City. Tanzania Journal of Health
 481 Research 16(4): 1–12.
 482

483 Oliveira, A. C. G., Seixas, A. S. S., Sousa, C. P. and Souza, C. W. O. 2006.
 484 Microbiological evaluation of sugarcane juice sold at street stands and juice
 485 handling conditions in São Carlos, São Paulo, Brazil. Cadernos de Saude
 486 Publica / Ministerio Da Saude, Fundacao Oswaldo Cruz, Escola Nacional de
 487 Saude Publica 22(5): 1111–1114.

488

489 Park, J. and Kim, M. 2013. Comparison of dry medium culture plates for
490 mesophilic aerobic bacteria in milk, ice cream, ham, and codfish fillet
491 products. Preventive Nutrition and Food Science 18(4): 269–72.

492

493 Pathak, A., Dwivedi, M., Laddha, N., Begum, R. and Joshi, A. 2013. DNA
494 isolation from fresh leaf tissue of *Tylophora indica* and *Bacopa monnieri*.
495 Environmental and Experimental Biology 11: 69–71.

496

497 Sabat, G., Rose, P. and Hickey, W. J. 2000. Selective and sensitive method
498 for PCR amplification of *Escherichia coli* 16S rRNA genes in soil. Applied and
499 Environmental Microbiology 66(2): 844–849.

500

501 Sharifa Ezat, W. P., Netty, D. and Sangaran, G. 2013. Paper review of factors,
502 surveillance and burden of food borne disease outbreak in Malaysia.
503 Malaysian Journal of Public Health Medicine 13(2): 1–7.

504

505 Suneetha, C., Manjula, K. and Depur, B. 2011. Quality assessment of street
506 foods in Tirumala. Asian Journal of Experimental Biological Sciences 2(2):
507 207–211.

508

509 Tambekar, D.H., Jaiswal, V.J., Dhanorkar, D.V, Gulhane, P.B. and Dudhane,
510 M.N. 2009. Microbial Quality and safety of street vended fruit juices : A case
511 study of Amravati city. Internet Journal of Food Safety 10: 72–76.

512

513 Tan, S. L., Lee, H. Y. and Mahyudin, N. A. 2014. Antimicrobial resistance of
514 *Escherichia coli* and *Staphylococcus aureus* isolated from food handler's
515 hands. Food Control 44: 203–207.

516

517 Ushiyama, M. and Iwasaki, M. 2010. Evaluation of Sanita-kun *E. coli* and
518 coliform sheet medium for the enumeration of total coliforms and *E. coli*.
519 Journal of AOAC International 93(1): 163-183.

520

521 Waku, S., Miyagi, M., Saito, R., Ueki, T., Baba, T. and Konoshita, M. 2013.
522 Microorganism culture sheet US 20130084624 A1. Retrieved on February
523 11, 2016 from Google Patents Website:
524 <http://www.google.com/patents/US20130084624>

525

526 World Health Organization (WHO) 1996. Essential safety requirements for
527 street-vended foods. Retrieved on March 10, 2016 from WHO Website:
528 <http://apps.who.int/iris/handle/10665/63265>

529

530 World Health Organization (WHO) 2003. Emerging issues in water and
531 infectious disease. Geneva, Switzerland: WHO Press, World Health
532 Organization. Retrieved on March 10, 2016 from WHO Website:
533 http://www.who.int/water_sanitation_health/emerging/emerging.pdf.

534

535 Yan, X., Fratamico, P. M., Bono, J. L., Baranzoni, G. M. and Chen, C. Y.

536 2015. Genome sequencing and comparative genomics provides insights on
537 the evolutionary dynamics and pathogenic potential of different H-serotypes
538 of Shiga toxin-producing *Escherichia coli* O104. BMC Microbiology 15(1): 83.
539
540 Yong, J. W. H., Ge, L., Ng, Y. F. and Tan, S. N. 2009. The chemical
541 composition and biological properties of coconut (*Cocos nucifera* L.) water.
542 Molecules 14(2): 5144–5164.
543
544

Table 1. Microbiological quality of sugarcane and coconut juice samples

Stall	Total Plate Count		Total Coliform Count		<i>Escherichia coli</i>		<i>Staphylococcus</i> spp.	
	Mean		Mean		Mean		Mean	
	(log CFU/ml)		(MPN/ml)		(MPN/ml)		(log CFU/ml)	
	Sugarcane	Coconut	Sugarcane	Coconut	Sugarcane	Coconut	Sugarcane	Coconut
A	2.49 ± 0.02 ^b	2.50 ± 0.05 ^a	93	150	240	93	2.62 ± 0.02 ^a	2.76 ± 0.02 ^a
B	2.63 ± 0.01 ^a	2.56 ± 0.01 ^a	>1100	>1100	240	460	2.28 ± 0.01 ^b	2.36 ± 0.01 ^b
C	2.32 ± 0.01 ^c	2.12 ± 0.01 ^b	>1100	43	240	<3.0	ND	ND

ND: Not Detected

Data are expressed as means ± standard deviation (n=3).

^{abc} values with different superscripts in the same column indicate significant difference ($P<0.05$).

552

553 Table 2. Bacterial load of associated food contact surfaces

Sample type / Stall	Total Plate Count (log CFU/ml)	Total Coliform Count (MPN/ml)	<i>Escherichia coli</i>		<i>Staphylococcus</i> spp.	
			MPN/ml	log CFU/cm ²	log CFU/ml	log CFU/cm ²
<i>Left hand</i>						
A	2.64 ± 0.04 ^b	240	43	ND	<2.40	<2.40
B	2.96 ± 0.02 ^a	460	240	ND	2.53 ± 0.01	2.63 ± 0.01
C	<2.40	23	23	ND	ND	ND
<i>Right hand</i>						
A	2.69 ± 0.06 ^b	240	43	ND	<2.40	<2.40
B	2.90 ± 0.02 ^a	460	240	<2.40	2.61 ± 0.04	2.69 ± 0.04
C	<2.40	23	23	ND	ND	ND
<i>Aprons</i>						
A	<2.40	<3.0	<3.0	ND	<2.40	<2.40
B	3.77 ± 0.02 ^a	43	23	ND	3.08 ± 0.01	3.11 ± 0.01
C	1.87 ± 0.02 ^b	93	43	<2.40	ND	ND
<i>Sugarcane juice extractors</i>						
A	<2.40	<3.0	<3.0	ND	ND	ND
B	4.13 ± 0.01 ^a	460	93	<2.40	2.46 ± 0.05	2.44 ± 0.01
C	3.91 ± 0.01 ^b	240	240	2.79 ± 0.01	ND	ND
<i>Coconut openers</i>						
A	<2.40	<3.0	<3.0	ND	2.62 ± 0.02 ^a	<2.40
B	2.91 ± 0.01 ^a	<3.0	<3.0	ND	2.28 ± 0.01 ^b	2.50 ± 0.01
C	2.86 ± 0.02 ^b	<3.0	<3.0	ND	<2.40	ND

554 ND: Not Detected
555 Data are expressed as means \pm standard deviation (n=3)
556 ^{ab} values with different superscripts in the same column indicate significant difference ($P<0.05$) for each type of food contact
557 surfaces

558

559 Table 3. Similarity of 16S rRNA gene sequences of samples based on the
560 NCBI database using BLAST analysis

Isolates	Similarity from BLAST	Identity	Accession
S3	<i>Escherichia coli</i> strain RM9387	99%	CP009104.1
S4	<i>Escherichia coli</i> strain RM9387	99%	CP009104.1
S6	<i>Escherichia coli</i> strain c164	99%	JQ781646.1
S9	<i>Escherichia fergusonii</i> strain E10	99%	KJ626264.1

561 Note: Isolate S3: Stall A sugarcane juice
562 Isolate S4: Stall B sugarcane juice
563 Isolate S6: Stall C sugarcane juice
564 Isolate S9: Stall C sugarcane juice extractor
565

566

567

568

569

570

571

572

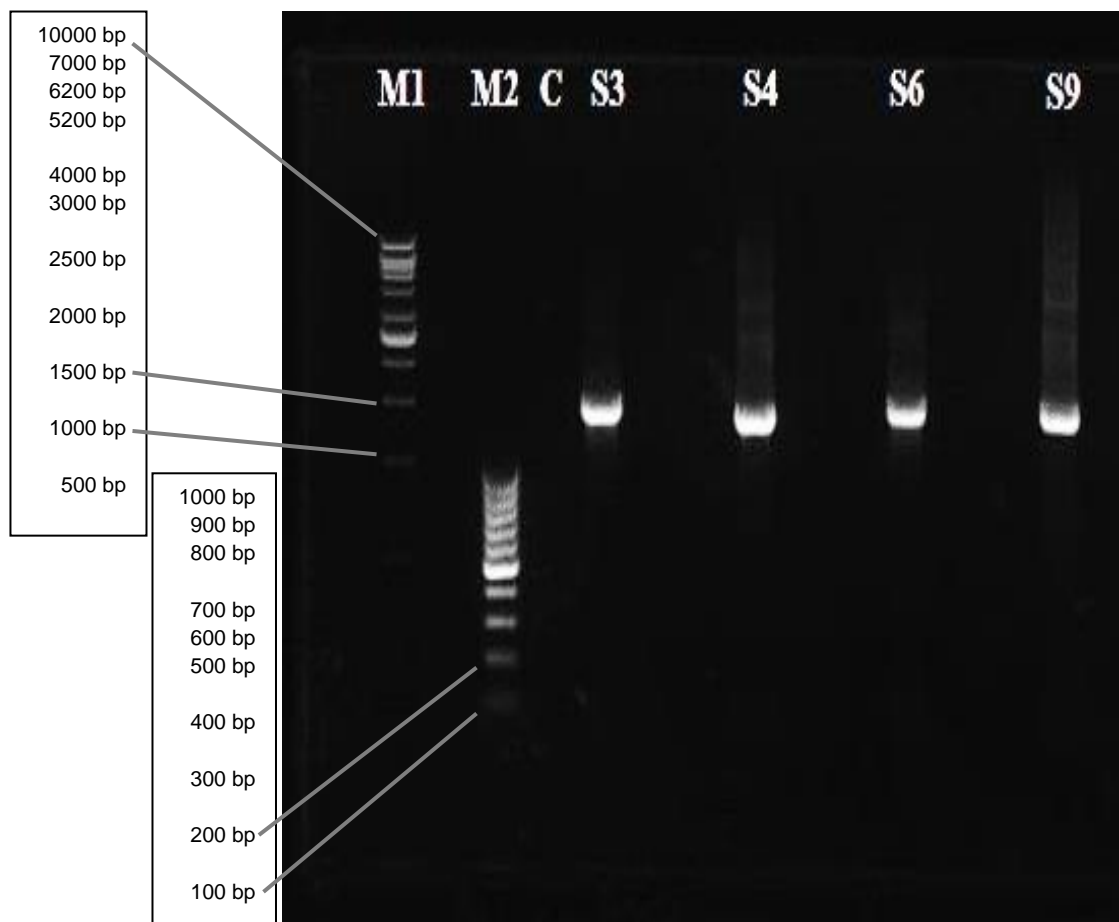
573

574

575

576

577



578

579 M1: 1 kb ladder; M2: 100 bp ladder; C: Negative control; S3: PCR product
580 from stall A sugarcane juice; S4: PCR product from stall B sugarcane juice;
581 S6: PCR product from stall C sugarcane juice extractor; S9: Stall C
582 sugarcane juice

583

584 Figure 1. PCR products visualised under UV illumination on 1.0% agarose
585 gel

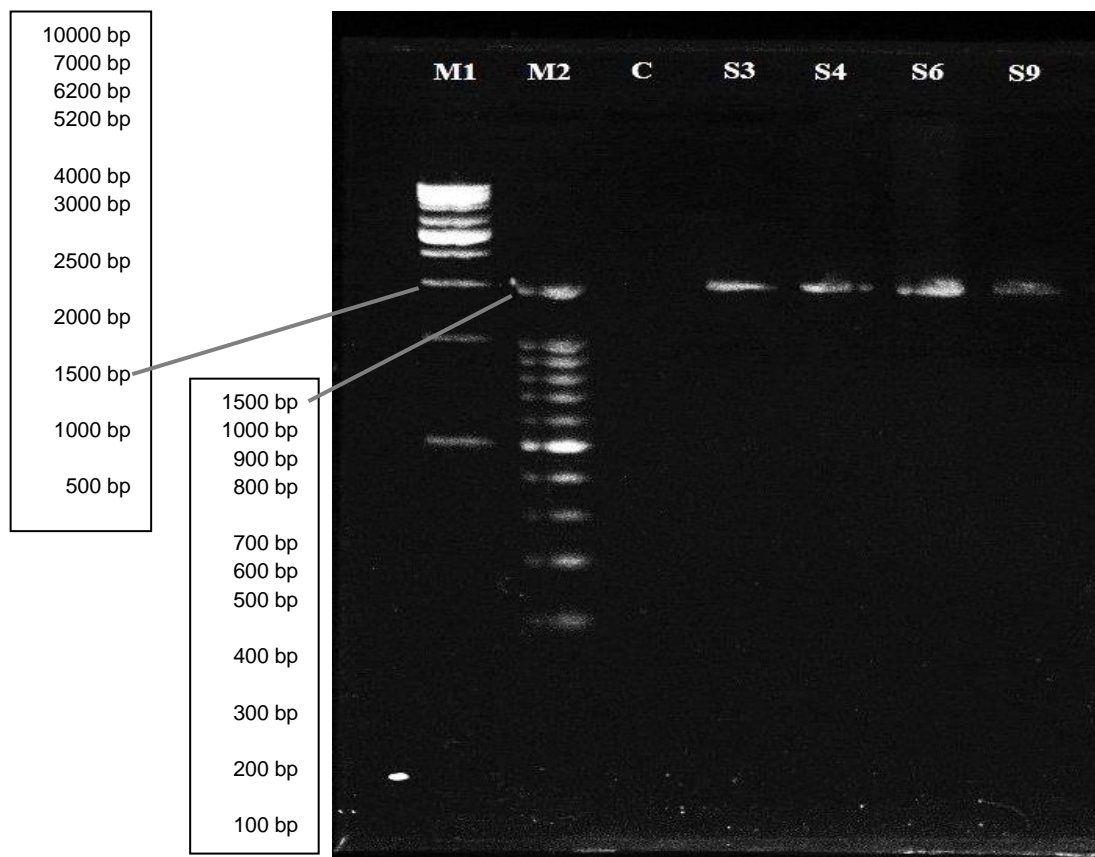
586

587

588

589

590



591

592 M1: 1 kb ladder; M2: 100 bp ladder; C: Negative control; S3: PCR product
 593 from stall A sugarcane juice; S4: PCR product from stall B sugarcane juice;
 594 S6: PCR product from stall C sugarcane juice extractor; S9: Stall C
 595 sugarcane juice

596

597 Figure 2. Purified PCR products visualised under UV illumination on 1.0%
 598 agarose gel

599